

CLAIMS

What is claimed is:

1. A live attenuated derivative of a pathogenic Enterobacteriaceae species, consisting essentially of

(a) a means for regulatable expression of a gene that encodes a regulatory protein, wherein expression of said regulatory protein in vivo causes synthesis of antigenic proteins that are conserved among Enterobacteriaceae; and

(b) a means for regulatable synthesis of a second antigen, wherein said second antigen ceases to be synthesized in vivo, exposing a carbohydrate antigen that is conserved among Enterobacteriaceae;

wherein said attenuated derivative has enhanced ability to induce cross protective immunity against Enterobacteriaceae.

2. The live attenuated derivative of claim 1, wherein said means of regulatable expression comprises substituting the promoter of said gene that encodes a regulatory protein with a regulatable promoter.

3. The live attenuated derivative of claim 2 wherein said regulatable promoter is the *araCP_{BAD}* repressor-activator-promoter system.

4. The live attenuated derivative of claim 3 wherein said carbohydrate antigen is an LPS O-antigen.

5. The live attenuated derivative of claim 4 wherein said means for regulatable synthesis comprises a mutation in a gene that encodes a product necessary for synthesis of LPS O-antigen.

6. The live attenuated derivative of claim 5, wherein said means for regulatable synthesis comprises a mutation in the *pmi* gene.

7. A method for inducing an immune response sufficient for protection against infection by Enterobacteriaceae species, said method comprising administering to an individual the live attenuated derivative of claim 1.

8. A live attenuated derivative of a pathogenic Enterobacteriaceae species, consisting essentially of

(a) a means for regulatable expression of a *fur* gene; and

(b) a mutation that renders a *pmi* gene inoperable,

wherein said attenuated derivative has enhanced ability to induce cross protective immunity against Enterobacteriaceae.

9. The live attenuated derivative of claim 8 wherein said means of (a) comprises substituting the *fur* promoter with a regulatable promoter.
10. The live attenuated derivative of claim 8, wherein said means of (a) comprises replacing the *fur* promoter with the *araCP*_{BAD} activator-repressor-promoter system.
11. The live attenuated derivative of claim 8 wherein said means of (a) comprises the Δ Pfur223::*araCP*_{BAD} genetic construction.
12. The live attenuated derivative of claim 8 wherein said mutation of (b) is a deletion mutation.
13. A method of inducing a cross-protective immune response against Enterobacteriaceae species, said method comprising administering to an individual the live attenuated derivative of any of claims 8-12.
14. A live attenuated derivative of a pathogenic Enterobacteriaceae consisting essentially of
 - (a) a means for regulatable expression of a first surface antigen, wherein said first surface antigen is conserved among Enterobacteriaceae; and
 - (b) a means for regulatable expression of a second surface antigen, wherein said second surface antigen is not conserved among Enterobacteriaceae, wherein up regulation of said first surface antigen and down regulation of said second surface antigen results in enhanced ability of said attenuated derivative to produce immunity against Enterobacteriaceae.
15. A vaccine comprising a live attenuated strain of *Salmonella*, wherein said live attenuated strain consists essentially of
 - (a) a mutation in a *pmi* gene that renders said *pmi* gene non functional; and
 - (b) a genetic construction that allows for regulatable expression of a *fur* gene, wherein said vaccine has enhanced ability to stimulate cross protective immunity against Enterobacteriaceae.
16. A method for inducing an immune response to Enterobacteriaceae comprising administering to an individual a live attenuated derivative of a pathogenic Enterobacteriaceae that is capable of colonizing the intestinal tract and reaching and persisting in the Gut Associated Lymphoid Tissue, and wherein expression of at least one conserved surface antigen is up regulated and at least one non-conserved surface antigen is down regulated in said attenuated derivative when said attenuated derivative is in the lymphoid tissue of the individual, wherein said live attenuated derivative has enhanced ability to stimulate cross protective immunity against infection by Enterobacteriaceae.

17. A vaccine comprising a live attenuated strain of *Salmonella*, wherein said live attenuated strain consists essentially of
- (a) a mutation that renders a *pmi* gene non functional; and
 - (b) a regulatable promotor operably linked to a *fur* gene wherein said *fur* gene is expressed when said attenuated strain is in the intestinal tract of an individual and said *fur* gene is not expressed when said attenuated strain is within internal tissues of an individual.
18. The vaccine of claim 17 wherein said regulatable promoter comprises the *araCP*_{BAD} activator-repressor-promoter system.
19. A live attenuated derivative of an Enteropathogenic bacteria consisting essentially of
- (a) a means for regulatable synthesis of LPS O-antigen side chains, wherein said O-antigen side chains are synthesized when said attenuated derivative is in the intestinal tract of an individual and are not synthesized when said attenuated derivative is within internal tissues of an individual; and
 - (b) a means for regulatable expression of a *fur* gene, wherein said *fur* gene is expressed when said attenuated derivative is in the intestinal tract of an individual and wherein said *fur* gene is not expressed when said attenuated derivative within internal tissues of an individual
- wherein said attenuated derivative has increased ability to induce cross protective immunity against infection from Enterobacteriaceae.
20. The live attenuated derivative of claim 19 wherein said means for regulatable synthesis comprises a mutation in a gene that encodes a product necessary for synthesis of LPS O-antigens.
21. The live attenuated derivative of claim 20 wherein said gene that encodes a product necessary for synthesis of LPS O-antigens is a *pmi* gene.
22. A live attenuated derivative of a *Salmonella typhimurium* comprising
- (a) a $\Delta P_{fur}::TTaraCP_{BAD}fur$ deletion-insertion mutation; and
 - (b) a Δpmi mutation
23. A recombinant bacterial strain consisting essentially of a means of regulatable expression of a virulence gene, wherein said regulatable expression of a virulence gene renders said bacterial strain attenuated while maintaining immunogenicity.
24. The recombinant bacterial strain of claim 23, wherein said means of regulatable expression comprises substituting the promoter for said virulence gene with the *araCP*_{BAD} repressor-activator-promoter system.
25. The recombinant bacterial strain of claim 24, wherein said virulence gene is a *fur* gene.

26. The recombinant bacterial strain of claim 25, wherein said bacterial strain is a strain of *Salmonella*.
27. The recombinant bacterial strain of claim 26, further comprising a Δpmi mutation.
28. A live attenuated derivative of a pathogenic *Enterobacteriaceae* species consisting essentially of a $\Delta P_{fur}::araCP_{BAD}fur$ genetic construction.
29. The live attenuated derivative of claim 28, wherein said species is *Salmonella*.